

=> s 13 and adaptor#
L9 0 L3 AND ADAPTOR#

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L10 1 L3 AND PRIMER#

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L10 ANSWER 1 OF 1 MEDLINE
AN 96132147 MEDLINE
DN 96132147 PubMed ID: 8580961
TI **Transposon** tagging of the maize **Glossy2** locus with the
transposable element **En/Spm**.
AU Tacke E; Korfhage C; Michel D; Maddaloni M; Motto M; Lanzini S; Salamini
F; Doring H P
CS Istituto Sperimentale per la Cerealicoltura, Sezione di Bergamo, Italy.
SO PLANT JOURNAL, (1995 Dec) 8 (6) 907-17.
Journal code: 9207397. ISSN: 0960-7412.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-X88779
EM 199603
ED Entered STN: 19960327
Last Updated on STN: 19990129
Entered Medline: 19960319
AB The **Glossy2** (G12) locus of maize is required for the formation of the
epicuticular wax layer of young plants. **gl2** mutant seedlings can be
visually identified because of their **glossy** leaf surface which is
different from the dull surface of wild-type seedlings. The G12 locus was
isolated by **transposon** tagging. Seven unstable mutations, **gl2-m2**
to **gl2-m8**, were induced in a parental strain carrying an active
transposable Activator (Ac) element in the unstable **wx-m7** allele.
Genetic tests on the **gl2-m2** allele indicated that it was not caused by the
Ac element but by the insertion of the **transposable** element
Enhancer/Suppressor-Mutator (En/Spm). A **Sa/l** restriction
fragment **segregating** with the **mutant phenotype**
was identified, by Southern analysis, using sequences from the En/Spm
element as a probe. Part of the fragment was cloned and was shown to carry
part of the unstable **gl2-m2** allele. These **gl2** sequences were used to
identify a genomic fragment carrying the wild-type allele and to isolate
its corresponding cDNA sequence. The predicted **Glossy2** protein consists of
426 amino acids. No similar amino acid sequence was found in protein data
banks and the biochemical function of the G12 gene product is still
unknown. The wild-type G12 transcript is found predominantly in juvenile
leaves. The transcript level in the leaves of seedlings homozygous for a
stable recessive **gl2-ref** allele is hardly detectable.
TI **Transposon** tagging of the maize **Glossy2** locus with the
transposable element **En/Spm**.
AB . . . their **glossy** leaf surface which is different from the dull
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Activator (Ac) element in the unstable **wx-m7** allele. Genetic tests on the
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Mutator (En/Spm). A **Sa/l** restriction fragment **segregating**
with the **mutant phenotype** was identified, by Southern
analysis, using sequences from the En/Spm element as a probe. Part of the
fragment was cloned.
CT . . . Support, U.S. Gov't, Non-P.H.S.
Alleles

Amino Acid Sequence

Base Sequence

Blotting, Northern

Cloning, Molecular

*Corn: GE, genetics

Corn: ME, metabolism

DNA Primers

***DNA Transposable Elements**

DNA, Complementary

DNA, Plant: IP, isolation & purification

DNA, Plant: ME, metabolism

Deoxyribonucleases, Type II Site-Specific

Enhancer Elements.

CN 0 (DNA Primers); 0 (DNA Transposable Elements); 0 (DNA, Complementary); 0 (DNA, Plant); 0 (Glossy2 protein); 0 (Plant Proteins); EC 3.1.21.- (endodeoxyribonuclease SalI); EC 3.1.21.4 (Deoxyribonucleases,

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DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ

<u>L12</u>	L10 and (primer\$1 near5 hybridiz\$5)	5	<u>L12</u>
<u>L11</u>	L10 and primer\$1 near5 hybridiz\$5	5	<u>L11</u>
<u>L10</u>	L9 and adapter	11	<u>L10</u>
<u>L9</u>	L8 and muta\$5	50	<u>L9</u>
<u>L8</u>	l6 and (transposable near5 element\$1)	50	<u>L8</u>
<u>L7</u>	(segregat\$3 or separat\$3) near5 phenotype\$1 near 10 muta\$5	0	<u>L7</u>
<u>L6</u>	(segregat\$3 or separat\$3) near5 phenotype\$1	416	<u>L6</u>
<u>L5</u>	(segregat\$3 or separat\$3) near5 phenotype\$1 nea5 muta\$5	0	<u>L5</u>
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<u>L3</u>	L2	0	<u>L3</u>
<u>L2</u>	L1 and phenotype\$1	0	<u>L2</u>
<u>L1</u>	5958738.pn.	2	<u>L1</u>

END OF SEARCH HISTORY

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1. [6479629](#). 18 Jun 01; 12 Nov 02. Maize histone deacetylases and their use. Baldwin; Donald Adelphi, et al. 530/324; 424/94.1 536/23.2 800/279. A61K038/00.

2. [6455297](#). 07 Aug 01; 24 Sep 02. Methods and compositions for regulating cell death and enhancing disease resistance to plant pathogens. Johal; Gurmukh S., et al. 435/232; 435/252.3 435/320.1 435/69.1 530/300 530/350 530/376 536/23.1 536/23.2. C12N009/88 C12N001/20 C12N015/00 C12P021/06 C07H021/04.

3. [6287843](#). 31 Mar 99; 11 Sep 01. Maize histone deacetylases and their use. Baldwin; Donald Adelphi, et al. 435/252.3; 435/419 435/6 536/23.2 800/279. C12N001/20 C12N005/04 C12Q001/68 C07H021/04 A01H001/00.

4. [6271439](#). 02 Mar 99; 07 Aug 01. Methods and compositions for regulating cell death and enhancing disease resistance to plant pathogens. Johal; Gurmukh S., et al. 800/279; 435/468 536/23.1 536/23.2 800/285 800/301 800/320.1. C12N015/29 C12N015/52 C12N015/09 C12N015/11 A01H005/00.

5. [WO 9941415 A1 AU 9926794 A](#). A new method to isolate and identify genes. ARBUCKLE, J A, et al. C07H021/00 C07H021/04 C12P019/34 C12Q001/68.

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Term	Documents
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PRIMER.DWPI,EPAB,JPAB,USPT.	73194
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PRIMERN.DWPI,EPAB,JPAB,USPT.	3
PRIMERO.DWPI,EPAB,JPAB,USPT.	3
(L10 AND (PRIMER\$1 NEAR5 HYBRIDIZ\$5)).USPT,JPAB,EPAB,DWPI.	5

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L12: Entry 3 of 5

File: USPT

Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6287843 B1
TITLE: Maize histone deacetylases and their use

Detailed Description Text (8):

A fragment of an HD nucleotide sequence that encodes a biologically active portion of an HD protein of the invention will encode at least 15, 25, 30, 50, 100, 150, 200, 250, 300, 350, 400, or 450 contiguous amino acids, or up to the total number of amino acids present in a full-length HD protein of the invention (for example, 458, 351, 439, 517, 432, 305, 302, 311, or 285 amino acids for SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, or 18, respectively). Fragments of an HD nucleotide sequence that are useful as hybridization probes for PCR primers generally need not encode a biologically active portion of an HD protein.

Detailed Description Text (9):

A fragment of an HD nucleotide sequence may encode a biologically active portion of an HD protein, or it may be a fragment that can be used as a hybridization probe or PCR primer using methods disclosed below. A biologically active portion of an HD protein can be prepared by isolating a portion of one of the HD nucleotide sequences of the invention, expressing the encoded portion of the HD protein (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded portion of the HD protein. Nucleic acid molecules that are fragments of an HD nucleotide sequence comprise at least 15, 20, 50, 75, 100, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, or 1,400 nucleotides, or up to the number of nucleotides present in a full-length HD nucleotide sequence disclosed herein (for example, 1826, 1475, 2019, 1943, 1576, 1283, 1191, 1245, or 1307 nucleotides for SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, or 17, respectively).

Detailed Description Text (12):

The proteins of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the histone deacetylase proteins can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (1985) Proc. Natl. Acad. Sci. USA 82:488-492; Kunkel et al. (1987) Methods in Enzymol. 154:367-382; U.S. Pat. No. 4,873,192; Walker and Gaastra, eds. (1983) Techniques in Molecular Biology (MacMillan Publishing Company, New York) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff et al. (1978) Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D.C.), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferred.

Detailed Description Text (13):

Thus, the genes and nucleotide sequences of the invention include both the naturally occurring sequences as well as mutant forms, likewise, the proteins of the invention encompass both naturally occurring proteins as well as variations and modified forms thereof. Obviously, the mutations that will be made in the DNA encoding the variant must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See, EP Patent Application Publication No. 75,444.

Detailed Description Text (36):

It is further recognized that the constructs of the invention may globally modulate gene activity or alternatively, may target particular regions of the chromosome. Assays are available for determining activity. See, generally, Lusser et al. (1997)

Science 277:88-91; Rundlett et al. (1996) PNAS 93:14503-14508; DeRubertis et al. (1996) Nature 384:589-591; Pazin et al. (1997) Cell 89:325-328; herein incorporated by reference. See also, Walton et al. (1993) Ann. Rev. Phytopathol. 31:275-303; Brosch et al. (1995) Plant Cell 7:1941-1950; Walton et al. (1985) Experientia 41:348-350; Yoshida et al. (1995) Bioessays 17:423; Taunton et al. (1996) Science 272:408-411; Pazin et al. (1997) Cell 89:325-328; Verreaultet et al. (1996) Cell 87:95-104; Kaufman et al. (1997) Genes Dev. 11:345-357; Parthun et al. (1996) Cell 87:85-94; Ciuffetti et al. (1995) Physiol. Mol. Pl. Pathol. 46:61-70; Rasmussen et al. (1988) Physiol. Mol. Pl. Pathol. 32:283-292; Ciuffetti et al. (1983) Biochem. 22:3507-3510; Wolf et al. (1990) Plant Sci. 70:127-137; Ach et al. (1997) Plant Cell 9:1595-1606. Additionally, function of the HD sequences can be elucidated by the characterization of mutants isolated by TUSC (Benson et al. (1995) Plant Cell 7:75-84; Mena et al. (1996) Science 274:1537-1540; U.S. patent application Ser. No. 08/835,638, which is a continuation of U.S. patent application Ser. No. 08/262,056) screening.

Detailed Description Text (58):

In preparing the expression cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and transversions, may be involved.

Detailed Description Text (61):

The present invention also provides isolated nucleic acids comprising polynucleotides, of sufficient length and complementarity to a gene of the invention to use as probes or amplification primers in the detection, quantitation, or isolation of gene transcripts. For example, isolated nucleic acids of the present invention can be used as probes in detecting deficiencies in the level of mRNA in screenings for desired transgenic plants, for detecting mutations in the gene (e.g., substitutions, deletions, or additions), for monitoring upregulation of expression or changes in enzyme activity in screening assays of compounds, for detection of any number of allelic variants (polymorphisms) of the gene, or for use as molecular markers in plant breeding programs. The isolated nucleic acids of the present invention can also be used for recombinant expression of polypeptides, or for use as immunogens in the preparation and/or screening of antibodies. The isolated nucleic acids of the present invention can also be employed for use in sense or antisense suppression of one or more genes of the invention in a host cell, tissue, or plant. Attachment of chemical agents, which bind, intercalate, cleave and/or crosslink to the isolated nucleic acids of the present invention can also be used to modulate transcription or translation. Further, using a primer specific to an insertion sequence (e.g., transposon) and a primer which specifically hybridizes to an isolated nucleic acid of the present invention, one can use nucleic acid amplification to identify insertion sequence inactivated genes of the invention from a cDNA library prepared from insertion sequence mutagenized plants. Progeny seed from the plants comprising the desired inactivated gene can be grown to a plant to study the phenotypic changes characteristic of that inactivation. See, Tools to Determine the Function of Genes, 1995 Proceedings of the Fiftieth Annual Corn and Sorghum Industry Research Conference (American Seed Trade Association, Washington, D.C., 1995). Additionally, non-translated 5' or 3' regions of the polynucleotides of the present invention can be used to modulate turnover of heterologous mRNAs and/or protein synthesis. Further, the codon preference characteristic of the polynucleotides of the present invention can be employed in heterologous sequences, or altered in homologous or heterologous sequences, to modulate translational level and/or rates.

Detailed Description Text (63):

Likewise, cDNA constructs can also be tagged with short peptides for rapid detection and manipulation of the enzyme, fused to specific DNA-binding domains for directed localization to reporter gene promoters, and mutated to adjust the functional characteristics of domains within HD.

Detailed Description Text (76):

To characterize HD function in planta, the Trait Utility System for Corn (TUSC) will be used to isolate maize lines containing HD loci, that have been disrupted by the

Mutator (Mu) transposable element. Briefly, Mu-saturated lines are crossed into inbred lines to create a collection of 40,000 F1 individuals. DNA samples isolated from these individuals are pooled and used as template DNA for PCR with a Mu element terminal inverted repeat (TIR) sequence primer in combination with HD specific primers. A DNA pool, containing a Mu element near the region encoded by the HD primer will produce a positive signal when the reaction is blotted and probed with the HD cDNA. The PCR reactions are repeated using the individual DNA samples that were part of the positive pool in order to identify F1 plants containing putative Mu-tagged HD alleles. See, Benson et al. (1995) Plant Cell 7:75-84 and Mena et al. (1996) Science 274:1537-1540.

Detailed Description Text (81):

The function of the HD and MSI genes in planta will be elucidated by the characterization of mutant alleles isolated by TUSC screening. This method has already proven useful in the isolation of ZmMSI1 disrupted alleles. HD and MSI specific primers can be designed that will amplify a number of gene family members, thus greatly reducing the number of PCR reactions required and allowing for the quick isolation of multiple alleles that can be characterized easily by genomic DNA blot and sequence analysis. The exact site of Mu insertion will be determined by sequencing genomic DNA amplified from wildtype (wt) and Mu-disrupted alleles using PCR methods. The expression patterns of all Mu-disrupted alleles will be compared to the basic patterns of expression of these genes in the wt plant to determine if any stable transcripts are present, thereby characterizing the nature of the mutation. Lines carrying the Mu-disrupted ZmMSI1 alleles for RNA isolation from tissues normally expressing ZmMSI1 are being propagated to determine if they are nullizygous in nature.

Detailed Description Text (82):

To alleviate problems of expressivity, an introgression series will be initiated with the introduction of each Mu-disrupted allele into a number of well-characterized inbred lines (W23, B73 and Mo17). At each generation, heterozygous mutants will also be self-fertilized to generate homozygous mutants, which can be assayed in the following studies. All Mu-disrupted alleles isolated will be crossed to generate pertinent double mutants, which may be more affected in these assays. Mutant plants under analysis will always be compared to control siblings, segregating as a result of this cross, carrying wt HD and MSI loci in the same genetic background.

Detailed Description Text (84):

Initial RNA profiling experiments have identified 91 maize genes that exhibit altered mRNA accumulation six hours after infection of hm mutant plants by C. carbonum TOX2.sup.-. Of the genes showing increased expression, 14 were unaffected by application of purified HC-toxin during the fungal infection. Thirty-four other induced genes, however, did not respond in the presence of HC-toxin and thus are candidates for components of the defense response pathway that are regulated directly or indirectly by histone deacetylase. Similar results were observed among genes repressed during C. carbonum TOX2.sup.- infection. This illustrates the consequences of inhibiting histone deacetylase on gene expression during a defense response and indicates HD regulation of that response may involve both transcription induction and suppression.

Detailed Description Text (87):

Similar to above, plants carrying Mu-disrupted alleles of HD and p48/MSI co-regulator proteins necessary for defense gene activation may show increased sensitivity to infectious (HC-toxin producing; TOX2.sup.+) strains of C. carbonum. One TUSC line known to have a disruption at one of the maize MSI genes has exhibited such sensitivity; while plants with the parental genotypes can resist C. carbonum TOX2.sup.+ some plants from this mutated line show delayed disease symptoms after four days of infection.

Detailed Description Text (89):

Morphologies of the vegetative and floral structures will be assessed compared to non-mutant sibling plants. As the Mu-background is reduced by outcrossing into inbred lines, it may be possible to detect characteristic phenotypes, that segregate with the tagged allele under investigation, indicating a role for these (genes in normal development.

Detailed Description Text (91):

With the HD family members in hand, the tools useful for biochemical dissection of the complexes within which they function can be generated. Differentially-tagged versions of full length HD and MSI proteins will be expressed in *E. coli*, the yeast *Pichia* or in insect cells using a baculovirus system. Methods are available in the art for these systems. These proteins will be used to test association in *in vitro* binding assays to generate polyclonal antibodies for future use in *in vitro* co-precipitation assays. An initial study has been performed using a maize MSI1 protein fused to the GST protein and expressed in *E. coli*. This construct was immobilized to chromatography resin, and *in vitro* transcribed and translated ZmHD proteins were radiolabeled and passed over the resin. Analysis of the protein fractions that were retained during chromatography shows that ZmHD1b and 1c, but not 1a, interact with ZmMSI1 in this system. Such immobilized protein chromatography assays (binding assays) will also be used in combination with alanine scan or domain targeted mutants to map sites of interaction between the MSI and HD proteins.

Other Reference Publication (13):

Rossi et al., Identification and Characterisation of an RPD3 homologue from Maize (*Zea mays* L.) that is able to Complement an *rpd3* Null Mutant of *Saccharomyces Cerevisiae*, *Mol. Gen. Genet.*, 1998, pp. 288-296, vol. 258.